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Association of single nucleotide polymorphism (SNP) markers in candidate genes and QTL regions with pork quality traits in commercial pigs☆,☆☆

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ABSTRACT

Numerous reports have described genetic markers or genomic regions (QTL) associated with pork quality and/or palatability but few validation studies have been reported. Therefore, 156 SNP markers from 45 candidate genes and eight QTL regions were analyzed for association with pork quality and palatability traits from 888 pork loins. Loins were collected at three slaughter facilities and selected to represent a wide range of pork color, pH and marbling. Phenotypic data recorded included objective and subjective measures of color and marbling, purge loss, shear force, and cooking loss. Data were analyzed with SAS PROC MIXED where loin was fit as a random effect. Results indicated some of the markers tested should be useful in industry, while others are not segregating in all populations or linkage disequilibrium between markers and causative genetic variation fluctuates among populations limiting their universal utility. Genes with the largest effects on pork quality were MC4R, IGF2, CAST and PRKAG3.

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1. Introduction

Numerous reports of genetic markers for pork quality have been published. These studies include both genome scans and candidate gene approaches implemented in populations ranging from experimental F2 populations using exotic breeds to standard commercial populations. Unfortunately, few associations have been validated in additional populations of commercial pigs. To date, the markers most consistently associated with pork quality include ryanodine receptor 1 (RYR1; Fujii et al., 1991; Leach, Ellis, Sutton, McKeith, & Wilson, 1996), protein kinase adenosine monophosphate-activated γ_3 subunit (PRKAG3; Milan et al., 2000; Ciobanu et al., 2001), MC4R (Kim, Larsen, Short, Plastow, & Rothschild, 2000a) and recently calpastatin (CAST; Ciobanu et al., 2004; Lindholm-Perry et al., 2009; Nonneman et al., 2011).

Genetic markers that are predictive of pork quality could be used for genetic selection programs or enable processors to determine the best market for specific pork products. Most genetic selection in commercial swine is conducted within specific commercial proprietary lines where markers specific to each line may be the most economical application of the technology. However, independent swine producers or pork processors do not have access to this information, and genetic markers that are the causative genetic variant (quantitative trait nucleotide, QTN) or in strong linkage disequilibrium with the QTN are needed. To determine a marker's utility in multiple commercial populations requires a broad sampling of market animals with relevant phenotypic data.

The objective of this study was to test markers in candidate genes and within reported QTL regions for associations with measures of pork quality in a group of pork samples collected at three different abattoirs harvesting commercial market hogs.

2. Material and methods

2.1. Samples

Loin selection and processing is discussed in greater detail by Moeller et al. (2010). Briefly, loins were sampled from three different commercial abattoirs during the fall and spring with a total of 20 different sampling dates. To ensure a broad sampling of commercial germplasm and farms, each day the loins selected were harvested over an eight hour timeframe. Tissue samples were available for DNA extraction from fresh boneless loins sampled from two facilities (222 and 219 loins each) while a third facility provided fresh loins (n = 223) as well as loins enhanced by injection of a solution intended to improve tenderness and juiciness (n = 224). In an attempt to uniformly represent the range of pork quality observed commercially, an initial classification of high, medium or low for muscle pH, Minolta

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Table 1
Descriptive statistics of traits measured before enhancement.

Trait	Number of records	Mean	Standard deviation	Minimum	Maximum
Minolta a*	906	17.26	1.40	11.70	21.02
Minolta b*	906	4.94	1.33	1.90	10.60
Minolta L*	906	52.90	4.34	40.91	67.50
Loin pH 24 h post-mortem	906	5.77	0.24	5.34	6.65
Color score	906	3.15	1.02	1.00	6.00
Marbling score	906	2.56	1.27	1.00	6.00
Intramuscular fat content	904	3.09	1.38	0.22	6.93

L* and marbling was determined. Loins were selected to uniformly fill all cells of a $3\times3\times3$ design; however, due to a strong negative correlation between Minolta L* and pH, cells containing loins with high pH and Minolta L* values as well as cells containing loins with low pH and Minolta L* values were not equally represented. No information was available on pre-harvest management of these pigs and sex determination was not attempted.

2.2. Phenotypic data

Description of procedures used to collect pork quality measurements were presented in Moeller et al. (2010). Briefly, whole boneless loins were collected approximately 24 h post-mortem, cut near the seventh rib and allowed to bloom for 10 min. Then loin pH and L*, a* and b* color measurements using a Minolta colorimeter were recorded. Subjective visual color and marbling scores (1 to 6 scale) were collected as outlined by the National Pork Producer Council (NPPC, 2000). A loin sample was obtained to measure intramuscular fat content (IMF) by an ether extract method (AOAC, 2007).

Enhanced loins were injected with solution prior to aging. Loins were then weighed, vacuum packaged and aged at 2 °C for 7 to 10 days. Loins were removed from packaging, weighed to determine loin purge, and sliced into 2.54 cm thick chops and frozen at −28.8 °C for storage.

Four frozen chops from each loin were used for Warner–Bratzler shear force determination. Each chop was weighed frozen and thawed to determine thaw purge and then one chop from each loin was selected to be cooked to 145, 155, 165 or 175° F (62.8, 68.3, 73.9 or 79.4 °C, respectively) internal temperature. Cooking time and final temperature was recorded along with cooked weight to determine cooking loss. Chops were cooled to 22 °C for 4 h. Six 1.27 cm diameter cores were removed from each chop parallel to the longitudinal orientation of the muscle fibers and sheared with a Warner–Bratzler shearing device. The average of all six cores was analyzed. Mean, range and standard deviation are presented for each phenotype in Tables 1 and 2.

2.3. Genotypic data

Candidate gene SNPs were selected from literature reporting polymorphisms within or near genes expected to affect pork quality and/or composition. Genes affecting composition were included as adiposity is highly correlated with intramuscular fat content. Genes were included even if no association had previously been shown to affect the targeted traits. A complete list of all candidate genes genotyped is presented in Table 3.

Eight QTL regions were targeted. A primary factor for inclusion was genomic regions with convincing evidence of QTL from a Landrace × Duroc F2 population reported by Rohrer, Thallman, Shackelford, Wheeler, and Koohmaraie (2005) and corroborated in other studies. Chromosome 6 was also studied due to numerous associations reported for pork quality despite not being identified by Rohrer et al. (2005). For each selected QTL region, at least six SNP markers were selected, where two SNPs spanned the region 5–10 cM prior to the QTL peak, two SNPs were located over the QTL peak and two SNPs spanned the region 5–10 cM after the peak. If sufficient SNPs were available for a region, markers were selected based on anticipated information content within commercial pig populations. However, for some regions all SNPs within the range were tested. The QTL regions studied are presented in Table 4.

Sequence information available in GenBank on pork quality candidate gene SNP markers were compiled in a file, along with SNP markers flanking QTL regions based on the current USMARC porcine linkage map and processed through MassARRAY Assay Designer 3.1.2.2 (Sequenom Inc., San Diego, CA) to group SNPs into assay groups of approximately 30 SNPs. Oligonucleotides used for each SNP assay are presented in Supplemental Table 1. Assays were run according to manufacturer's protocols, analytes detected with mass spectrometry and genotypes called using MassARRAY TYPER 3.4 software (Sequenom Inc., San Diego, CA). Manual evaluation of all scored genotypes was performed. Assays that failed to provide a sufficient number of genotypes or where their genotypic distributions were clearly inconsistent with the Hardy–Weinberg equilibrium ($X^2_{2df}>20.0$; $p<4.5\times10^{-5}$) were eliminated from the study. Assays with low minor allele frequency (MAF<0.05) or that mildly deviated from the Hardy–Weinberg equilibrium ($6.0<X^2_{2df}<20.0$; $0.05>p>0.000045$) were left in the study, but their results should be considered with caution.

2.4. Statistical analyses

Data were analyzed using SAS version 9.2 (Cary, NC). The PROC MIXED procedure was used for all measurements. For traits recorded prior to enhancement (a*, b*, L*, pH, IMF, color and marbling scores) the model included fixed effects for PLANT, DATE and GENOTYPE and LOIN as a random effect. The analyses of purge loss included a fixed effect for ENHANCEMENT as well as the previous effects. Each SNP marker was analyzed independent of all other markers. Haplotype analyses were not attempted as genomic regions tested were too broad (20 or more cM).

There were four measurements for each loin for traits measured after freezing. The statistical model for thaw purge loss included the fixed effects of PLANT, DATE and ENHANCEMENT, LOIN was considered

Table 2
Descriptive statistics of traits measured after enhancement.

Trait	Natural loins					Enhanced loins				
	Number of records	Mean	Standard deviation	Minimum	Maximum	Number of records	Standard mean	Deviation	Minimum	Maximum
Purge loss-aging, %	674	1.96	1.91	0	10.62	227	3.84	1.78	1.08	19.96
Purge loss-Thawing, %	676	2.83	1.32	0	7.86	227	0.78	0.48	0	3.63
Cooking loss-62.8 °C	675	9.65	0.41	0.97	3.41	227	5.56	1.57	2.46	12.41
Cooking loss-68.3 °C	676	10.59	3.17	0.02	21.6	227	6.07	1.75	3.14	15.83
Cooking loss-73.9 °C	676	12.76	3.41	0	23.61	227	7.02	2.06	2.73	15.92
Cooking loss-79.4 °C	674	15.08	4.27	0	36.57	225	8.64	3.19	0	19.4
Shear force-62.8 °C	678	2.51	0.6	1.26	4.97	227	1.67	0.41	0.97	3.41
Shear force-68.3 °C	672	2.64	0.76	1.23	6.84	227	1.65	0.43	1	3.45
Shear force-73.9 °C	676	2.75	0.78	1.24	7.02	227	1.62	0.37	0.88	3.31
Shear force-79.4 °C	676	2.88	0.85	1.46	6.43	225	1.72	0.42	1.04	3.55

Table 3
Candidate genes evaluated for associations with pork quality traits.

Gene symbol	Gene name	Citation
ACACA	Acetyl coenzyme A carboxylase a	Munoz et al. (2007)
ADP	Adiponectin	AJ849536[a]
APOB	Apolipooprotein B	Jiang and Gibson, (1999)
ASIP	Agouti signaling protein	Kim, Kim, Dekkers, & Rothschild, 2004b
CAST	Calpastatin	Ciobanu et al. (2004)
CSTB	Cystatin B	Russo et al. (2002)
CTSB	Cathepsin B	Russo et al. (2002)
CYP21	Steriod 21 hydroxylase	Knoll, Cepica, Stratil, Nebola, and Dvorak (1998)
CYP2E1	Cytochrome p450 2E1	Skinner, Doran, McGivan, Haley, and Archibald (2005)
DECR1	Mitochondrial 2,4 dienoyl CoA reductase	Clop et al. (2002)
DLK1	Delta-like homolog 1	Kim, Choi, and Beever, (2004a)
FABP3	Fatty acid-binding protein-heart	Gerbens, Rettenberger, Lenstra, Veerkamp, and te Pas, (1997)
FASN	Fatty acid synthase	Munoz et al. (2007)
FHL3	Four-and-a-half LIM-only protein 3	Zuo et al. (2004)
FMO1	Flavin containing mono-oxygenase 1	Glenn et al. (2007)
FMO3	Flavin containing mono-oxygenase 3	Glenn et al. (2007)
FTO	Fat mass and obesity associated gene	Fontanesi et al. (2008a)
GAA	Alpha acid glucosidase	Fontanesi, Davoli, Nanni-Costa, and Russo (2003)
GH	Growth hormone	Larsen and Nielsen (1997); Kirkpatrick, Huff, and Casas-Carrillo (1993)
GHR	Growth hormone receptor	DQ38803[a]
GYS1	Glycogen synthase	te Pas et al. (2003)
HSD11B1	Beta hydroxysteroid dehydrogenase	Otieno, Bastiaansen, Ramos, and Rothschild (2005)
HSL	Hormone sensitive lipase	Harbitz, Langset, Ege, Hoyheim, and Davies, (1999)
IGF2	Insulin-like growth factor 2	Van Laere et al. (2003)
LDHA	Lactate dehydrogenase	Fontanesi et al. (2003)
LDLRRP1	Low density lipoprotein receptor related protein 1	AF526393[a]
LPL	Lipoprotein lipase	Lei et al. (2004)
MC4R	Melanocortin 4 receptor	Kim et al. (2000a)
MC5R	Melanocortin 5 receptor	Kim, Marklund, and Rothschild (2000b)
MEF2A	Myocyte enhancer factor 2A	Larsen et al. (1999)
MYF6	Myogenic factor 6	Wyszynska-Koko et al. (2006)
MYH4	Myosin heavy chain 2B	Davoli, Fontanesi, Braglia, and Russo (2003a)
MYOD1	Myogenic differentiation antigen 1	Urbanski and Kuryl, (2004)
PKLR	Pyruvate kinase, liver and red blood cell	Knoll, Stratil, Moser, and Geldermann (2000)
PKM2	Pyruvate kinase, muscle 2	Fontanesi et al. (2003)
PPARG	Peroxisome proliferator activated receptor g 1	Grindflek et al. (2004)
PPARGC1A(PGC1)	Peroxisome proliferator activated receptor g coactivator 1	Erkens, Rohrer, Van Zeveren, and Peelman (2009)
PRKAG3	Protein kinase adenosine monophosphate-activated g_3 subunit	Milan et al. (2000); Ciobanu et al. (2001)
RYR1	Ryanodine receptor 1	Fujii et al. (1991)
SDHD	Succinate dehydrogenase complex, subunit D	Guimaraes et al. (2007)
SFRS1	Splicing factor arginine/serine rich 1	Wang, Wang, Zhu, Yang, and Li (2005)
TGFB1	Transforming growth factor b	Kopecny et al. (2004)
TGFB1R	Transforming growth factor receptor b	Shimanuki et al. (2005)
TNNT3	Skeletal muscle troponin T3	Davoli et al. (2003b)
TYR	Tyrosinase	Okumura et al. (2005)

[a] GenBank Accession numbers (www.ncbi.nlm.nih.gov).

a random effect and GENOTYPE was nested within LOIN. Cooking loss and shear force used a similar model with the addition of COOKING_TIME, COOKED_TEMPERATURE and THAW_PURGELOSS as covariates. These additional covariates were included as they independently explained significant proportions of phenotypic variation in preliminary analyses. Nominal p-values for each test are reported. Due to the large number of statistical tests conducted, only those with a nominal significance of $p<0.01$ are presented, but all tests of significance are available in the Supplemental materials. Experiment-wise significant associations of $p<0.05$ were determined using a Bonferroni adjustment resulting in a nominal $p<2.87\times10^{-5}$. Genotypic variation of a specific SNP was calculated using the allele frequencies of each allele and estimates of a and d from the solutions to the statistical model based on an equation by Falconer (1981). Percentage of phenoptyic variation explained by a SNP was computed by dividing genotypic variation in that SNP by total phenotypic variation and multiplied by 100.

3. Results

A total of 201 SNP assays were run, but 45 were eliminated from the study (22 monomorphic, 8 failed and 15 gave inconsistent genotypic distributions). Probabilities for F-ratios testing association of

Table 4
QTL regions targeted for validation with SNP markers.

Position[a]	Traits	Citation
1:0–15	Intramuscular fat	Rohrer et al., 2005; Sanchez et al., 2007
1:60–90	Pork color, intramuscular fat and pH	Malek et al., 2001; Rohrer et al., 2005; Liu et al., 2007
2:0–15	Pork color, moisture and pH	Rohrer et al., 2005; Stearns et al., 2005
2:60–80	pH and shear force	Lee et al., 2003; Rohrer et al., 2005; Stearns et al., 2005; van Wijk et al., 2006; Liu et al., 2007
5:50–65	Pork color, cooking loss and pH	Malek et al., 2001; Rohrer et al., 2005
6:50–86	Pork color, intramuscular fat and pH	Grindflek et al., 2001; Malek et al., 2001; Yue et al., 2003; van Wijk et al., 2006
15:44–60	Pork color, intramuscular fat, pH and shear force	Malek et al., 2001; Nii et al., 2005; Rohrer et al., 2005; van Wijk et al., 2006; Liu et al., 2007; Sanchez et al., 2007
17:30–70	Pork color, intramuscular fat and juiciness	Malek et al., 2001; Rohrer et al., 2005

[a] Position is listed as chromosome:range in cM based on the current USMARC swine linkage map.

Table 5
Nominally significant ($p<0.01$) associations detected between SNP markers and pork quality traits.

Marker name	Trait	Probability[a]	LL[b]	LL se	HL	HL se	Percent of phenotypic variation	SSC	Position[c]
6193.1	Marbling score	3.06E−03	0.38	0.13	0.38	0.12	1.4	1	59.51
6193.1	Shear force	9.00E−04	−0.18	0.05	−0.04	0.05	0.9	1	59.51
26055.1	Loin purge	6.23E−03	0.26	0.74	0.54	0.17	0.6	1	59.51
26055.2[d]	Minolta b*	1.73E−03	−1.19	1.08	−0.48	0.14	1.0	1	59.51
24185_1[e]	Color score	1.17E−03	0.40	0.11	0.13	0.10	2.1	1	60.20
24185_1[e]	Loin pH	4.36E−04	0.10	0.03	0.02	0.02	2.3	1	60.20
24185_1[e]	Minolta b*	**3.84E−06**	−0.59	0.12	−0.21	0.10	2.6	1	60.20
24185_1[e]	Minolta L*	3.17E−04	−1.81	0.47	−0.62	0.39	2.4	1	60.20
15335_1	IMF	**2.76E−05**	−0.60	0.13	−0.12	0.10	2.4	1	75.70
15335_1	Marbling score	2.95E−05	−0.53	0.12	−0.06	0.09	2.3	1	75.70
11585_1[d]	Cooking loss	4.10E−03	3.69	2.13	4.25	2.12	0.4	1	77.25
11585_1[d]	Loin pH	8.40E−03	−0.43	0.23	−0.35	0.24	1.1	1	77.25
11585_1[d]	Thaw purge	**2.24E−05**	2.94	0.78	2.63	0.78	0.8	1	77.25
MC4R_1426	Color score	8.61E−04	−0.36	0.10	−0.29	0.09	1.7	1	82.50
MC4R_1426	IMF	5.44E−03	0.42	0.14	0.17	0.12	1.2	1	82.50
MC4R_1426	Loin pH	7.46E−03	−0.07	0.02	−0.05	0.02	1.2	1	82.50
MC4R_1426	Minolta L*	1.25E−03	1.50	0.42	1.11	0.38	1.5	1	82.50
MC5R	Minolta b*	6.50E−03	−0.34	0.12	−0.15	0.12	0.8	1	82.50
IGF2NC_1[e]	Color score	4.61E−03	−0.46	0.18	−0.27	0.18	1.3	2	0.70
IGF2NC_1[e]	Minolta b*	7.06E−03	0.53	0.19	0.37	0.20	0.8	2	0.70
IGF2NC_2[e]	Color score	2.90E−03	0.45	0.17	0.20	0.08	1.4	2	0.70
IGF2NC_3[e]	Color score	4.71E−03	0.45	0.18	0.19	0.08	1.3	2	0.70
IGF2NC_3[e]	Minolta a*	8.15E−03	0.68	0.22	0.08	0.09	0.9	2	0.70
IGF2NC_6[e]	Color score	8.90E−04	0.51	0.16	0.19	0.08	1.8	2	0.70
IGF2NC_6[e]	Minolta a*	2.20E−03	0.67	0.19	0.08	0.09	1.2	2	0.70
IGF2NC_6[e]	Minolta b*	1.23E−03	−0.57	0.17	−0.17	0.08	1.2	2	0.70
IGF2NC_6[e]	Minolta L*	5.88E−04	−2.38	0.66	−0.64	0.31	1.8	2	0.70
12329_1	Loin pH	8.27E−04	0.24	0.07	0.04	0.02	1.2	2	12.7
44785_814	Minolta L*	8.62E−03	−1.59	0.54	−0.50	0.30	1.0	2	64.80
27516_1	Loin pH	6.87E−03	0.07	0.02	0.04	0.02	0.6	2	68.40
27516_1	Minolta L*	9.16E−03	−1.12	0.42	−0.26	0.38	1.0	2	68.40
21726_2[e]	Loin pH	7.70E−03	0.07	0.02	0.03	0.02	1.0	2	70.00
21726_2[e]	Marbling score	9.42E−03	−0.35	0.13	0.00	0.09	1.0	2	70.00
21726_2[e]	Thaw purge	1.80E−03	−0.23	0.08	0.06	0.06	0.5	2	70.00
65587_237	Marbling score	9.77E−03	−0.38	0.13	−0.19	0.09	1.1	2	75.50
23795_1[e]	Loin purge	4.47E−03	0.27	0.17	−0.17	0.16	1.0	2	83.60
23795_1[e]	Shear force	**1.49E−05**	0.21	0.05	0.05	0.05	1.3	2	83.60
41642_192	Shear force	1.00E−03	0.14	0.10	0.14	0.04	0.8	2	83.60
41642_408	Shear force	6.00E−04	−0.12	0.10	0.02	0.10	0.8	2	83.60
41646_595	Shear force	3.00E−04	−0.12	0.10	0.04	0.10	0.9	2	83.60
41646_874	Shear force	8.20E−03	−0.14	0.05	−0.12	0.05	0.5	2	83.60
41650_975	Shear force	2.50E−03	−0.13	0.10	0.00	0.10	0.7	2	83.60
41658_290	Shear force	5.00E−04	−0.09	0.10	0.07	1.00	1.0	2	83.60
17179_1	Cooking loss	4.00E−03	−0.51	0.18	−0.20	0.21	0.4	5	57.70
17179_1	Shear force	1.80E−03	0.00	0.05	−0.13	0.04	0.8	5	57.70
44017_483	Marbling score	7.84E−03	−0.39	0.12	−0.19	0.11	1.2	5	62.70
44360_103	Marbling score	9.24E−03	0.37	0.12	0.14	0.11	1.1	5	64.70
44360_178	IMF	5.61E−03	0.88	0.31	0.67	0.32	1.1	5	64.70
MYF6	IMF	1.88E−03	−0.50	0.16	−0.24	0.16	1.4	5	139.80
MYF6	Marbling score	5.08E−03	−0.42	0.15	−0.21	0.15	1.1	5	139.80
HSL	Loin purge	7.04E−03	−3.27	1.21	−3.50	1.21	2.4	6	75.00
RYR1[d]	Loin purge	1.14E−03	−1.66	0.51	–	–	0.9	6	76.36
RYR1[d]	Shear force	**1.55E−10**	−1.01	0.16	–	–	2.2	6	76.36
SSC6_414848_198	Minolta b*	5.66E−04	0.16	0.11	0.32	0.08	1.2	6	77.4
SSC6_414848_198	Minolta L*	7.97E−03	0.62	0.42	1.00	0.32	1.1	6	77.4
7281_1	Loin purge	1.83E−03	−0.37	0.17	−0.54	0.15	1.2	6	78.50
7281_2	Loin pH	2.50E−03	0.05	0.04	0.06	0.02	1.4	6	78.50
FHL3	Minolta a*	4.09E−03	0.19	0.33	−0.14	0.34	1.0	6	80.00
26725_1	IMF	3.77E−03	−0.50	0.15	−0.33	0.14	1.4	6	83.70
DLK1	IMF	7.51E−03	0.46	0.18	0.21	0.18	1.2	7	82.50
SDHD860	Minolta L*	4.63E−03	1.33	0.41	0.38	0.35	1.2	9	53.00
FMO1	Loin purge	7.29E−03	−0.56	0.18	−0.39	0.17	0.9	9	78.81
FASN1254	Shear force	2.20E−03	0.19	0.06	0.10	0.04	0.8	12	2.00
FASN3189	Shear force	6.30E−03	−0.17	0.06	−0.07	0.06	0.6	12	2.00
GH_200	IMF	8.73E−03	1.67	0.96	1.29	0.96	1.3	12	32.00
ADP	Color score	9.69E−04	−0.26	0.21	−0.32	0.09	1.9	13	60.00
ADP	Minolta L*	2.40E−03	0.93	0.80	1.14	0.33	1.3	13	60.00
23887_1[e]	Cooking loss	7.00E−03	0.46	0.48	0.84	0.49	0.5	15	58.60
23887_1[e]	Loin pH	1.80E−03	−0.06	0.05	−0.11	0.05	1.5	15	58.60
23887_1[e]	Minolta b*	5.40E−03	−0.16	0.25	0.09	0.25	0.8	15	58.60
23887_1[e]	Minolta L*	3.02E−03	0.73	0.96	1.69	0.96	1.3	15	58.60
23298.2	Color score	9.25E−03	0.05	0.21	−0.20	0.21	1.2	15	60.84
23298.2	Minolta b*	2.61E−03	−0.20	0.22	0.09	0.23	1.0	15	60.84
23298.2	Minolta L*	3.22E−03	−0.06	0.86	1.05	0.88	1.4	15	60.84

Table 5 (*continued*)

Marker name	Trait	Probability[a]	LL[b]	LL se	HL	HL se	Percent of phenotypic variation	SSC	Position[c]
26435.1	Minolta b*	7.54E−03	−0.88	0.42	−0.65	0.42	1.0	15	60.84
27510.1	Thaw purge	9.90E−03	2.17	0.78	0.13	0.11	0.4	15	63.60
PRK_89	Color score	5.68E−05	0.60	0.14	0.47	0.14	2.2	15	84.60
PRK_89	Cooking loss	9.80E−03	−0.78	0.29	−0.61	0.29	0.5	15	84.60
PRK_89	Loin pH	**9.81E−06**	0.15	0.03	0.10	0.03	2.5	15	84.60
PRK_89	Minolta b*	**6.52E−06**	−0.62	0.15	−0.35	0.15	1.8	15	84.60
PRK_89	Minolta L*	**5.92E−06**	−2.71	0.56	−2.05	0.58	2.5	15	84.60
PRKQTN	Color score	1.99E−03	0.64	0.18	0.55	0.18	1.7	15	84.60
PRKQTN	Cooking loss	**1.46E−09**	−2.16	0.37	−2.16	0.37	2.5	15	84.60
PRKQTN	Loin pH	**1.57E−07**	0.23	0.04	0.23	0.04	3.9	15	84.60
PRKQTN	Marbling score	4.45E−03	0.73	0.22	0.63	0.22	1.4	15	84.60
PRKQTN	Minolta a*	4.01E−03	−0.71	0.22	−0.71	0.22	1.1	15	84.60
PRKQTN	Minolta b*	3.58E−04	−0.75	0.19	−0.76	0.19	1.4	15	84.60
PRKQTN	Minolta L*	8.21E−03	−2.33	0.75	−2.16	0.74	1.2	15	84.60
PRKQTN	Thaw purge	1.30E−03	−0.48	0.14	−0.51	0.14	0.6	15	84.60
11717_1[d]	Minolta b*	6.73E−03	0.59	0.22	–	–	0.6	17	38.71
15247.1	IMF	5.31E−03	0.31	0.22	0.31	0.10	1.2	17	38.80
15247.1	Marbling score	6.47E−03	0.23	0.20	0.28	0.09	1.1	17	38.80
15247.1	Minolta b*	4.42E−03	0.41	0.18	0.22	0.08	0.8	17	38.80
42813_84	Shear force	1.30E−03	0.01	0.06	−0.13	0.04	0.8	17	45.90
6533.1[e]	Shear force	2.00E−04	−0.22	0.06	−0.23	0.06	0.8	17	66.00
6533.1[e]	Thaw purge	9.00E−04	−0.33	0.09	−0.25	0.09	0.5	17	66.00

[a] Probabilities in bold exceed the experiment-wise error rate of $P<0.05$.

[b] Genotypic effects and standard errors for individuals homozygous for the low molecular weight analyte (LL) or heterozygous (HL). The effect for individuals homozygous for the high molecular weight analyte was 0.00. A — in the HL column indicates that there were no HH animals and the HL class was set to 0.00.

[c] Genetic map position based on the current USMARC swine linkage map.

[d] Minor allele frequency was <0.10.

[e] Genotypic distribution significantly ($p<0.05$) deviated from the Hardy–Weinberg equilibrium expectations.

genotype at each of the 156 SNP markers with each of the 11 traits are in Supplemental Table 2. Results are reported in Table 5 along with estimated genotypic effects for 96 associations significant at the nominal $p<0.01$ threshold. Approximately one-third of these associations were with SNP markers that deviated from the Hardy–Weinberg equilibrium ($p<0.05$) or had low minor allele frequencies (MAF<0.05). Ten associations were significant after a Bonferroni adjustment to the threshold ($p<2.87\times10^{-5}$).

Number of associations for each trait ranged from four (Minolta a*) to 14 (Warner–Bratzler shear force). In general, the fewest association were detected for measures of water holding capacity and the most associations were for measures of color (excluding Minolta a*) and shear force. Chromosomes 2 and 15 had the most associations detected (25 and 22, respectively) largely due to SNP markers in CAST and PRKAG3.

4. Discussion

4.1. Validation of QTL regions and candidate genes

Seven of eight QTL regions investigated had markers with significant ($p<0.01$) associations with the reported phenotype. The only QTL region for which no associations were detected was the QTL at SSC 1, 0–15 cM where previous research had found QTL for intramuscular fat content (Rohrer et al., 2005; Sanchez et al., 2007). In addition, no association with markers at SSC2, 0–15 cM were detected with juiciness or cooking loss as identified by Stearns et al. (2005) or Rohrer et al. (2005), respectively. Similarly, no associations were detected with markers at SSC5, 50–65 cM, for color or pH as reported (Malek et al., 2001; Rohrer et al., 2005).

Associations of markers within the region of SSC1, 60–90 cM for color, pH and intramuscular fat traits (Liu et al., 2007; Malek et al., 2001; Rohrer et al., 2005) were confirmed in these data. There appears to be two causative factors for color and pH traits segregating in this broad region of chromosome 1. A marker located at 60.2 cM was significantly associated with these traits (24185_1) along with the SNP in MC4R located at 82.5 cM, but no significant associations were detected with color for the eight SNPs between 60.2 and 82.5 cM. However, for marbling traits the most significant associations were detected for a marker in the middle of this interval at 75.7 cM (15335_1). This region contained the candidate genes melanocortin-4 receptor (MC4R) and melanocortin-5 receptor (MC5R). The MC4R 298Asn allele was associated with lower pH in the current study which concurs with Duroc and Landrace populations studied by Piorkiwska et al. (2010), even though no effect of this polymorphism was detected in Large White pigs (Piorkiwska et al., 2010) or in crossbred pigs examined by Van den Maagdenberg et al. (2007). In addition, we found the MC4R 298Asn allele was associated with greater intramuscular fat as reported by Schwab et al. (2009) and Van den Maagdenberg et al. (2007). However, results were inconsistent with other studies of MC4R and intramuscular fat (Piorkiwska et al., 2010; Stachowiak, Szydloski, Obarzanek-Fojt, & Switonski, 2005).

Considerable evidence was found to support a QTL segregating at the top of SSC 2 affecting pork color, with all of the associations located in the insulin-like growth factor 2 (IGF2) gene. As the IGF2 QTN discovered by Van Laere et al. (2003) could not reliably be genotyped on the Sequenom system, it cannot be determined if these associations are due to the IGF2 QTN reported or other genetic variation segregating in this region. The results in the IGF2 region with color traits concurs with the results of Hueven et al. (2009) where they found a QTL for Minolta values. The only association of this region and pH was at position 12.7 cM (12329_1).

The results clearly indicate that at least one QTN within SSC2, position 60 to 80 cM, affecting shear force was segregating in these samples. All of the significant associations were with SNP markers residing in the CAST gene. The most significant marker was with a SNP detected by a random approach to find SNP markers in the swine genome (23795_1) that has previously been associated with shear force (Rohrer et al., 2007). Some of the markers reported by Ciobanu et al. (2004) were significantly associated with shear force as well as other SNP discovered in the gene (Lindholm-Perry et al., 2009; Meyers, Rodriguez-Zas, & Beever, 2007). Determining which CAST SNP would be most predictive for general populations is difficult as results seem to vary across populations (Lindholm-Perry et al., 2009; Nonneman et al., 2011 and Rohrer et al., 2007), but the markers developed by Nonneman et al. (2011) which used these loin samples

would indicate their markers are more predictive than ones used in this study. Incomplete linkage disequilibrium with the causative SNP or multiple QTN in CAST could cause conflicting results across populations. Significant associations were detected with this region for pH, color and thaw purge loss.

The QTL identified by Rohrer et al. (2005) for cooking loss at SSC 5, position 50 to 65 cM was detected in these samples with a marker (17179_1) located at position 57.7 cM. In addition to the cooking loss association, this region also had significant associations with marbling (62 to 65 cM) and shear force (57.7 cM).

Previous associations of markers on SSC 6 with pH, color, IMF and loin purge (Grindflek, Szyda, Liu, & Lien, 2001; Malek et al., 2001; van Wijk et al., 2006; Yue et al., 2003) were supported by associations with markers located in a narrow region of SSC 6, 77 to 79 cM. This region contains RYR1 and there was a very low incidence of the deleterious allele (frequency 0.006). Despite the low allele frequency, RYR1 was associated with purge loss and shear force. The effect of the deleterious 1843T allele in the RYR1 gene on water holding capacity is well documented (Carlson, Christian, Kuhlers, & Rasmusen, 1980; Kukoyi, Addis, McGrath, Rempel, & Martin, 1981; Krzecio et al., 2005); however, its effect on tenderness is not. As the RYR1 mutation affects Ca^{+2} transport and the calpain protease system is Ca^{+2} concentration dependent (Koohmaraie, 1992), the observed effect on shear force could be due to reduced calpain activity in animals possessing the 1843T allele. Additional candidate genes were located in this region and significant associations were detected with HSL (loin purge) and FLH3 (Minolta a*).

Based on these results, all of the significant associations on SSC 15 are likely due to PRKAG3 SNP as either the Rendement Napole (RN) QTN, denoted as R200Q, or the T30N SNPs were the most significant for every trait analyzed. In these samples, the markers were in linkage equilibrium as determined by a chi-square test ($X^2_{4df} = 4.05$; $p = 0.40$) on the genotypic distributions. Subsequent analyses fitting both SNPs as fixed effects revealed the F-ratio for both markers was greater and the p-value was at least half that relative to single marker analyses (data not shown). This implies that both SNPs are affecting pork quality traits independently and should be considered in selection decisions. An attempt was made to genotype the I199V SNP reported by Ciobanu et al. (2001), but the genotypes called appeared unreliable based on a Hardy–Weinberg equilibrium test ($X^2_{2df} = 68.65$) and associations observed were less significant than the ones reported for R200Q or T30N (data not shown). Our findings support the deleterious effects of the PRKAG3 200Q allele on color, pH and water holding measurements reported by Milan et al. (2000). Furthermore, the associations between the PRKAG3 T30N mutation and pork quality were approximately two-fold larger, but in the same direction, as those reported by Ciobanu et al. (2001) and Fontanesi, Scotti, Buttazzoni, Davoli, and Russo (2008b). The larger estimated effects were actually more similar to those reported for the PRKAG2 I199V mutation and may be due to linkage disequilibrium between these two SNP markers.

QTL detected on SSC 17 for color, juiciness and IMF traits (Malek et al., 2001; Rohrer et al., 2005) were supported by marker associations in the current study. Associations with marbling were found at 38.8 cM, Minolta b* at 38.7 to 38.8 cM and thaw purge loss at 66.0 cM. In addition, two associations with shear force were detected at 45.9 and 66.0 cM.

Phenotypic correlations between pork color, pH and purge loss imply that the following candidate gene associations not discussed above concur with previous findings. Flavin containing mono-oxygenase 1 (FMO1) was associated with loin purge loss (previous reported associations with Minolta values; Glenn, Ramos, & Rothschild, 2007) and succinate dehydrogenase complex, subunit D (SDHD) was associated with Minolta L* (previously associated with pH; Guimaraes, Rothschild, Ciobanu, Stahl, & Lonergan, 2007). The remaining associations with candidate genes were inconsistent with the gene's function and/or other results. The genes adiponectin (ADP) and fatty acid synthase (FASN) were expected to affect lipid metabolism but were associated with color and tenderness traits, respectively. Myogenic factor 6 (MYF6) and delta-like 1 homolog 1 (DLK1) are expected to affect muscle growth, yet both were associated with IMF in the present study. Growth hormone (GH) has previously been associated with carcass weight (Wyszynska-Koko et al., 2006) but was associated with IMF in the present work. Many of the candidate gene SNP had not been directly tested for associations with measurements of pork quality collected in this study; rather, the authors had suggested that they may be useful due to the gene's function. Therefore, it is not surprising that only a few congruent associations were observed in this sample of commercial pork loins.

4.2. Sample collection effects

As the samples studied were collected at three different facilities with multiple collection dates/location, they should represent a broad section of US commercial market hogs. This feature greatly reduced the extent of linkage disequilibrium present and requires markers to be physically closer to the QTN to detect significant associations. Unfortunately, when samples are collected at a commercial facility, pedigree information is typically not available. Without pedigree information and DNA samples from parents, analyzing haplotypes or imprinting effects are difficult. For genes that have previously been shown to have parent of origin effects (DLK1 and IGF2), the results from the current study should be reviewed with caution. Pedigree information facilitates fitting an animal model which can account for additional additive genetic variation yielding results with greater significance levels and fewer false positive associations (Rohrer et al., 2007).

4.3. Hardy–Weinberg equilibrium

The Hardy–Weinberg equilibrium was tested to assess accuracy of genotype scoring. Unfortunately, the Hardy–Weinberg equilibrium assumes that all animals are from a single random mating population. As the sampling procedure attempted to maximize the number of populations represented and commercial market hog production relies heavily on terminal crosses (hybrid animals resulting from non-random mating), we only eliminated 15 assays with extreme deviations from expectations ($p < 4.5 \times 10^{-5}$).

Twenty of the reported assays deviated from the Hardy–Weinberg equilibrium at $p < 0.05$. Of the 20 reported assays, some of these deviations are likely artifacts of the Sequenom genotyping system when assaying 30 or more SNPs simultaneously. Occasionally the system will have difficulty scoring one of the three possible genotypes for a SNP when the system is challenged at this level. A conservative approach was taken in calling genotypes to ensure that the genotypes analyzed were accurate and the analyses were correct. However, this results in unequal call rates across genotypes, leading to deviations from the Hardy–Weinberg equilibrium. Typically these assays had approximately 50 fewer scored genotypes than assays in equilibrium and parameters for many of the unscored animals put their data point on the borderline of the genotype deficient in number of animals relative to Hardy–Weinberg expectations. Thus, if a majority of the unscored animals were placed in the deficient genotypic class, then the Hardy–Weinberg equilibrium would be achieved.

However, one region that was clearly not in the Hardy–Weinberg equilibrium was the IGF2 region. All four of the IGF2 SNPs scored deviated from the Hardy–Weinberg equilibrium ($p < 0.05$), yet averaged over a 98% call rate. There was a deficiency of heterozygotes for all four SNPs. Heterozygote deficiencies can occur when different populations with widely different allele frequencies are incorrectly grouped into a common population, known as a Wahlund effect. As these samples were collected across populations, this would imply that populations varied considerably in allele frequencies, possibly

due to different selection objectives. As these four markers had clear genotype calls, high call rates, are closely linked and all indicated a deficiency in heterozygotes, this phenomenon definitely appears to be a function of the animals sampled (Wahlund effect) and not the genotypic calling procedure.

5. Conclusions

Some of the markers tested in this study appear to be very robust and are predictive of phenotype in most swine populations. The most definitive groups of markers are those within PRKAG3, where they are predictive of pH, color and water holding capacity traits of pork products. The IGF2 markers are another group of markers where the effects were quite consistent on all color traits measured; however, validating its effect on composition or mode of inheritance was not possible in this study. The MC4R SNP also appears to be associated with color and pH. Finally, while there is considerable evidence to indicate a QTL is segregating for tenderness near CAST, the most predictive SNP markers for use in all populations remains to be determined.

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